Micellization of Cationic Gemini Surfactants with Various Counterions and Their Interaction with DNA in Aqueous Solution

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The micellization of six cationic gemini surfactants with various counterions, $[C_{12}H_{25}(CH_3)_2N(CH_2)_6N(CH_3)_2C_{12}H_{25}]X_2$, designated as $C_{12}C_6C_{12}X_2$, with $X = F^-$, Cl^-, Br^-, Ac^-, NO_3^-, and $^1/_2SO_4^{2-}$ in aqueous solutions has been investigated by isothermal titration microcalorimetry (ITC) and conductivity measurements. The interaction of these surfactants with DNA in aqueous solutions has also been investigated by isothermal titration microcalorimetry. The critical micelle concentration (*CMC*) and the degree of micellar ionization (α), the critical aggregation concentration (*CAC*), the saturation concentration (C_2) of the aggregation, and the associated thermodynamic parameters were determined. The nature of the counterion significantly affects the processes of both micellization and aggregation. The trends for aggregation basically follow the Hofmeister (lyotropic) series, but the pattern of the variation of the enthalpy of aggregation often revealed a more complex behavior. Among the counterions examined, SO_4^{2-} is the most effective anion for decreasing the *CMC* (or *CAC*). Both aggregation processes are mainly entropy-driven since the values of the entropy changes multiplied by temperature are much larger than the absolute values of the enthalpy changes. The binding of micelles to DNA is strongly dominated by the positive entropy gain on release of the small counterions from the micelles and from DNA. The interaction of all of the surfactants with DNA was dependent on the DNA concentration and may be associated with each micelle interacting with more than one DNA molecule.

Introduction

Cationic lipid−DNA interactions have been the subject of many studies over the past few decades because they are of interest both in fundamental science and in biotechnological applications, for example, as efficient nonviral artificial reagents for gene delivery in gene therapy.[1,2] Properties such as structure, thermodynamics, and morphology of cationic lipid−DNA complexes have been widely investigated by a variety of methods.[3−11] These studies show that variations in length, degree of unsaturation, flexibility, and chemical structure of the lipid chain and the nature of the counterion can each have a large effect on the interaction process and transfection efficiency. However, despite such extensive studies, the interaction processes and their mechanisms are poorly understood and many unsolved questions about the relationship of the structure of the lipid with the properties of these complexes remain. Cationic gemini (dimeric) surfactants, structurally similar to the more complicated cationic lipids used for transfection studies, have received increasing attention as simpler models for transfection complexes.[12−17] A deeper study of these systems could be beneficial to the better comprehension of DNA complexation.

Gemini surfactants[18] comprise two single-chain surfactant moieties joined by a hydrocarbon spacer group. Most gemini surfactants so far investigated are bisquaternary ammonium bromide compounds having the general structure $[C_mH_{2m+1}(CH_3)_2N(CH_2)_sN(CH_3)_2C_nH_{2n+1}]Br_2$, designated as $C_mC_sC_nBr_2$, where *m* and *n* indicate the numbers of carbon in the side alkyl chains and *s* indicates the numbers of carbon in the spacer, respectively.[18−29] Their behaviors at interfaces and in aqueous solutions have been well explored and this work has been summarized in several review articles.[30−32]

It is well-known from studies on single-chain surfactants that the counterion has a strong influence on the critical micelle concentration (*CMC*), aggregation number, and size and shape of aggregates of ionic surfactant systems.[33−39] There have, however, been few reports on bisquaternary ammonium-type gemini surfactants with counterions other than bromide[40−42] and surprisingly few systematic studies of the effect of the counterion. Zana et al.[40] showed that substitution of chloride for bromide had a large effect on the viscosity, *CMC*, degree of micelle ionization (α), and Gibbs free energy (ΔG) of micellization of gemini surfactants. Oda and co-workers[41−42] used transmission electron microscopy (TEM) to study a series of low-molecular-weight gelators based on gemini surfactants, $C_{16}C_2C_{16}X_2$, with $X_2 = 2Br^-$, L-malate, D-glucarate, 2D-gluconate, and *L*-, *D*- and *meso*-tartrates, and found that covalent interactions between the charged headgroups and chirality in the counterions are critical factors for determining the morphologies of aggregates and their ability to gel various solvents.

In the present work, we focus on the effect of counterion on the micellization of several cationic gemini surfactants with different counterions and on their interaction with DNA using isothermal titration microcalorimetry and conductivity measurements. These surfactants have the structure, $[C_{12}H_{25}(CH_3)_2N(CH_2)_6N(CH_3)_2C_{12}H_{25}]X_2$, designated as $C_{12}C_6C_{12}X_2$, with X

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$= F^-$, Cl^-, Br^-, Ac^-, NO_3^-, and $^1/_2SO_4^{2-}$, where X denotes the monovalent counterion neutralizing the ammonium headgroups.

Experimental Section

Materials. The $C_{12}C_6C_{12}X_2$ with various counterions were synthesized and purified as follows. The gemini surfactant with bromide counterion was synthesized according to the method of Menger and Littau.[21] A large excess of dodecyldimethylamine (prepared by direct reaction of bromododecane with dimethylamine in methanol) was reacted with the dibromohexane in warm acetone (<40°C) until precipitation of the hexanediyl-1,6-bis(dodecyldimethylammonium bromide) was complete. The product was recrystallized several times from a mixture of ethanol and ethyl acetate. The $C_{12}C_6C_{12}X_2$ with F^-, Cl^-, Ac^-, NO_3^-, and $^1/_2SO_4^{2-}$ were prepared by ionic exchange. The gemini surfactant with bromide was resolved in water, then a large excess of basic ionic exchange resin (Dowex) was added to replace bromide with hydroxide OH^-. At the end of the exchange, typically complete in less than an hour, the solution was filtered and the appropriate acid was added until the solution was neutralized as precisely as possible. The resulting solution was freeze-dried to give the corresponding salt. The dry surfactant was repeatedly recrystallized at least twice until no minimum was observed in the surface tension.

Low-molecular-weight salmon sperm DNA from Fluka BioChemika Co. was used without further purification. DNA stock solution was prepared by dissolving dried DNA in water with no buffer. The concentration of DNA was determined by measurements of UV absorbance assuming the molar extinction coefficient $\epsilon_{260} = 6.6 \times 10^3\ M^{-1}\ cm^{-1}$ expressed in nucleotide phosphates. Triply distilled water was used in all experiments.

Conductivity Measurements. Electrical conductivity was used to determine the critical micelle concentration (*CMC*) and the degree of micelle ionization (α) of the $C_{12}C_6C_{12}X_2$ surfactants. Conductivities of the surfactant solutions were measured as a function of concentration with a Model 4320 Conductivity Meter (Jenway, England). All measurements were performed in a double-walled glass container with the temperature being controlled by circulation of water at 298.15 ± 0.05 K. A fraction of a solution about 10 times more concentrated than the estimated *CMC* of the surfactant was successively added to 10 mL of water. Sufficient time was allowed between consecutive additions for the system to equilibrate.

Isothermal Titration Microcalorimetry. The calorimetric measurements were conducted using a TAM 2277−201 microcalorimetric system (Thermometric AB, Järfälla, Sweden) with a stainless steel sample cell of 1 mL at 298.15 K. The cell was initially loaded with 0.5 mL water or DNA solution and the concentrated gemini surfactant solutions of 5 mM were injected into the stirred sample cell in 40−45 portions of 5−10 μL using a 500−μL Hamilton syringe controlled by a Thermometric 612 Lund Pump. The interval between two injections was 15 min, which was sufficiently long for the signal to return to the baseline. The system was stirred at 50 rpm with a gold propeller. During the whole titration processes of gemini surfactants into DNA, the change of pH was less than 0.15. Raw data curves were integrated using Digitam 4.1 software as described in the instrument manual. The accuracy of the calorimeter was periodically calibrated electrically and verified by measuring the dilution enthalpies of concentrated sucrose solution (0.985 mol L^{-1}). The resulting value was always in excellent agreement ($\pm$ 1%) with the literature value.[43] All experiments were repeated twice, and the reproducibility was within $\pm$ 4%.

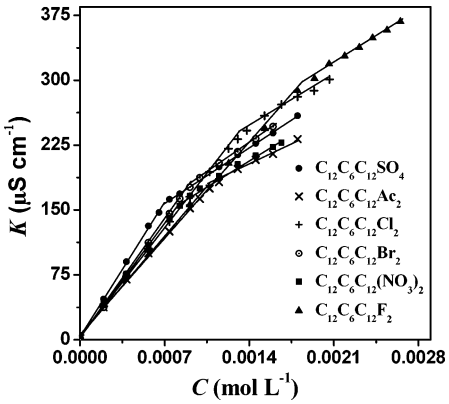

Figure 1. Variations of the conductivity κ of aqueous solutions with the concentration C of $C_{12}C_6C_{12}X_2$ surfactants at 298.15 K.

Results and Discussion

Micellization of the $C_{12}C_6C_{12}X_2$. We first used microcalorimetry and conductivity measurements to study the effect of counterion on the micellization for the $C_{12}C_6C_{12}X_2$ surfactants. It should be noted that most previous studies on the effect of counterion in surfactant solutions have used added electrolyte. However, adding electrolyte is completely different from substitution of the counterion.[33−34,39,44] This is partly because the increase in ionic strength has a major effect and partly because the incomplete removal of the original counterion may still affect the surface and aggregation properties.

1. Electrical Conductivity. Figure 1 shows the variations of the electrical conductivity κ of aqueous solutions with the concentration C of the $C_{12}C_6C_{12}X_2$ surfactants at 298.15 K. There clearly exist breaks corresponding to the *CMC* in these plots, where the electrical conductivity of surfactant solutions changes with surfactant concentration at different rates below and above the *CMC*,[20] and the sharpness of the breaks helps to confirm the purities of the surfactant samples. The degree of ionization of the micelles, α, was taken to be the ratio of the values of $d\kappa/dC$ above and below the *CMC*, and then was calculated from the slopes of the two straight lines in these regions.[45,46] The degree of ionization α can also be replaced by the degree of counterion association to micelle (β) obtained by the relationship $1 - \alpha$. Both α and β can reflect the ability of counterion binding on micelles. The larger the α, that is, the smaller the β, means the weaker ability of counterion binding to micelles.

Values of *CMC* and α determined from the electrical conductivity measurements are listed in Table 1. As expected, there is a pronounced effect of counterion on both *CMC* and α, and their values increase in the sequence $SO_4^{2-} < NO_3^- < Br^- < Ac^- < Cl^- < F^-$. When allowance is made for the surfactant ion concentration in the sulfate, which is double the value of the *CMC* as given, this order correlates well with the Hofmeister (lyotropic) series of anions for cationic surfactant,[47] $NO_3^- > Br^- > Cl^- > Ac^- > F^- \approx SO_4^{2-}$, with only Cl^- and Ac^- being interchanged. The position of an anion in this series is considered to depend on its hydrated radius or polarizability and charge. A decreasing hydrated radius of the anion is usually accompanied by an increasing polarizability. A large polarizability should enhance the binding of the counterion at the micellar surface and also decrease the electrostatic repulsion between the headgroups of the surfactant molecules, accordingly increasing the tendency to aggregate and lowering both *CMC* and α. It is therefore no surprise that the correlation of the *CMC*s with the Hofmeister series is so good.

TABLE 1: Critical Micelle Concentration, Degree of Micelle Ionization and Thermodynamic Parameters for the $C_{12}C_6C_{12}X_2$ Surfactants at 298.15 K Using Electrical Conductivity and Calorimetric Measurements

	electrical conductivity		microcalorimetry			
	CMC (mmol L^{-1})	α	*CMC* (mmol L^{-1})	ΔH_{mic} (kJ mol^{-1})	ΔG_{mic}[a] (kJ mol^{-1})	$T\Delta S_{mic}$[b] (kJ mol^{-1})
SO_4^{2-}	0.68 ± 0.03	0.40 ± 0.02	0.53 ± 0.03	7.3 ± 0. 6	-29.9	37.2
NO_3^-	0.89 ± 0.03	0.41 ± 0.02	0.83 ± 0.04	−6.5 ± 0. 4	-36.3	29.8
Br^-	0.98 ± 0.03	0.42 ± 0.02	0.89 ± 0.03	−5.1 ± 0. 3	-35.6	30.5
Ac^-	1.10 ± 0.03	0.44 ± 0.02	1.01 ± 0.03	−4.8 ± 0. 5	-34.3	29.5
Cl^-	1.33 ± 0.03	0.49 ± 0.02	1.30 ± 0.11	−0.9 ± 0.4	-31.6	30.7
F^-	1.84 ± 0.03	0.54 ± 0.02	1.83 ± 0.04	−4.1 ± 0. 5	-28.4	24.3

[a] Calculated using $\Delta G_{mic} = RT(1 + 2\beta)\ln cmc - RT\ln 2$, and $\Delta G_{mic} = RT(1 + \beta)\ln(cmc/2)$ for gemini surfactants with monovalent counterions and with divalent counterion, respectively,[50] where *cmc* is expressed in molarity of each alkyl chain, twice as much as the *CMC* expressed in molarity of surfactant; and β was obtained according to the relationship $\beta = 1 - \alpha$. [b] Calculated from $\Delta G_{mic} = \Delta H_{mic} - T\Delta S_{mic}$.

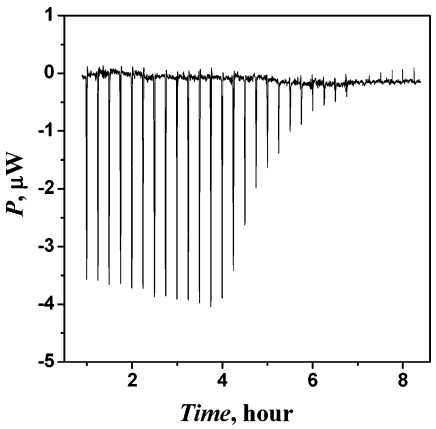

Figure 2. Raw data isothermal calorimetric titration curve of $C_{12}C_6C_{12}Ac_2$ into pure water at 298.15 K.

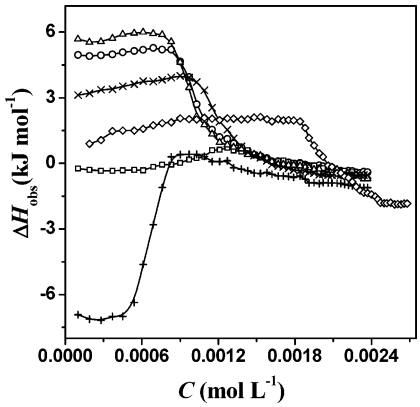

Figure 3. Variations of the observed enthalpies (ΔH_{obs}) of dilution of the $C_{12}C_6C_{12}X_2$ surfactants into water with the final concentration (*C*) of surfactant at 298.15 K. The initial concentrations of surfactants are all 5 mM: (○) $C_{12}C_6C_{12}Br_2$; (△) $C_{12}C_6C_{12}(NO_3)_2$; (×) $C_{12}C_6C_{12}Ac_2$; (◇) $C_{12}C_6C_{12}F_2$; (□) $C_{12}C_6C_{12}Cl_2$; (+) $C_{12}C_6C_{12}SO_4$.

2. Microcalorimetry. A typical experimental isothermal calorimetric titration curve for the dilution of $C_{12}C_6C_{12}Ac_2$ into pure water at 298.15 K is presented in Figure 2, which shows the thermal power *P* as a function of time *t*. The observed enthalpies (ΔH_{obs}) were obtained by the integral of the area under the calorimetric peaks.

The calorimetric titration curves of the variations of the observed enthalpies with the final concentration (*C*) of the $C_{12}C_6C_{12}X_2$ surfactants at 298.15 K are given in Figure 3, where the data points are the experimentally observed enthalpies per mole of surfactant. The dilution curves in pure water are all sigmoidal in shape and each curve can be subdivided into two concentration regions separated by a transition region associated with micelle formation, corresponding to *CMC*. When the final dilution concentration is below *CMC*, the enthalpy change results from the breakup of the added micelles and from the further dilution of the monomer solution. When the final dilution concentration is above *CMC*, only the micelle solution is diluted and ΔH_{obs} is close to zero. The *CMC*s were determined from the intersections of the two lines extrapolated from the slope as surfactant is initially added and the slope just above *CMC* of the plot of the observed enthalpies of dilution against surfactant concentration.[48] The enthalpies of micellization (ΔH_{mic}) can be determined from the difference at the *CMC* between the observed enthalpies of the two linear segments of the plots[49] as shown in Figure 4. The Gibbs free energies of micellization (ΔG_{mic}) can be calculated from *CMC* and β following the standard procedure in the literature.[50] The entropies of micellization (ΔS_{mic}) can then be derived from ΔH_{mic} and ΔG_{mic}. There was an excellent agreement between *CMC* values obtained from microcalorimetry and conductivity, as can be seen from the complete set of thermodynamic parameters listed in Table 1.

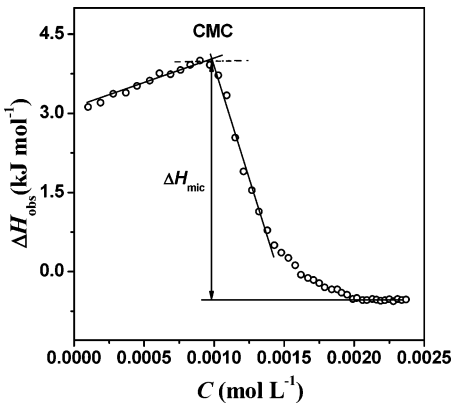

Figure 4. Determination of the *CMC* and ΔH_{mic} from the calorimetric titration curve of $C_{12}C_6C_{12}Ac_2$ from dilution into pure water at 298.15 K.

The enthalpies of micellization are generally expected to be exothermic, as observed for the $C_{12}C_6C_{12}X_2$ surfactants with X = F^-, Cl^-, Br^-, Ac^-, and NO_3^- (the titrations of these into pure water at 298.15 K are all endothermic). However, for $C_{12}C_6C_{12}SO_4$, the titration process is exothermic and the enthalpy of micellization is endothermic, and for $C_{12}C_6C_{12}Cl_2$, ΔH_{mic} is small and close to zero. For the monovalent counterions, the values of ΔG_{mic} follow the same order as the Hofmeister series (except for the inversion of Cl^- and Ac^-) becoming less negative in the direction of F^-, as would be expected from the *CMC*s. However, the values of $-\Delta H_{mic}$ and $-T\Delta S_{mic}$ for the Cl^- are distinctly anomalous with ΔH_{mic} being much less exothermic than the other members of the series and ΔS_{mic} much larger than F^-. Of these three halide ions, fluoride is very strongly hydrated and bromide should not be hydrated

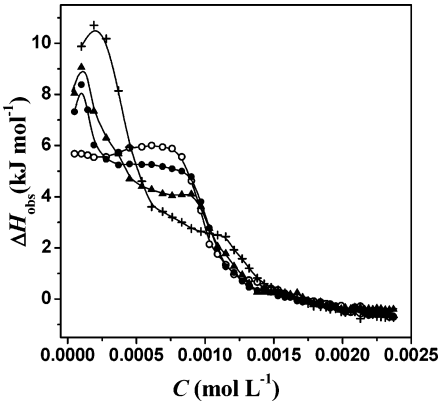

Figure 5. Calorimetric titration curves of $C_{12}C_6C_{12}(NO_3)_2$ into water (○), 0.33 mM DNA (●), 0.65 mM DNA (▲), and 1.63 mM DNA (+) at 298.15 K.

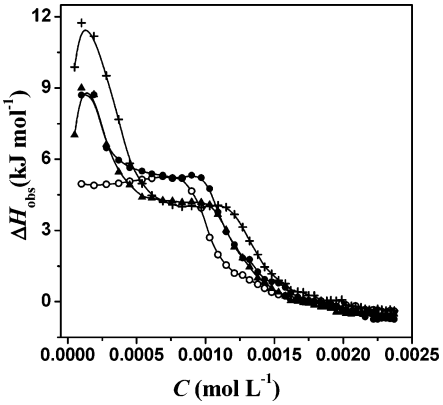

Figure 6. Calorimetric titration curves of $C_{12}C_6C_{12}Br_2$ into water (○), 0.33 mM DNA (●), 0.65 mM DNA (▲) and 1.63 mM DNA (+) at 298.15 K.

to any significant extent. Chloride is, however, hydrated, as observed by neutron diffraction.[51] However, its transport properties show that its water of hydration is much less strongly bound than in the fluoride ion. It is then plausible that the doubly charged environment of the gemini headgroups can dehydrate the chloride to some extent, but not the fluoride. This would lead to an endothermic contribution to the enthalpy and a gain in entropy from the released water molecules, which is exactly the effect observed. This then suggests that the anomalous endothermic enthalpy of micellization of the sulfate may have a similar origin. The sulfate ion should be strongly hydrated but with a lower hydration number than the chloride. The strong electric field in the doubly charged gemini surfactant ion may be able to dislodge some of this water of hydration. The enthalpy required to do this will be higher than for the chloride and will be offset by a lower gain of entropy because fewer water molecules are involved. The reason the gemini ion may do this for the sulfate but not for the fluoride could be due to the much greater distance of the hydration water from the center of the ion in the sulfate leading to a better match of the bare ion to the dimensions of the gemini headgroup region.

Aggregation of the $C_{12}C_6C_{12}X_2$ with DNA. The calorimetric titration curves of the variations of the observed enthalpies (ΔH_{obs}) of the $C_{12}C_6C_{12}X_2$ diluted into DNA solutions with the final concentration (C) of surfactant at 298.15 K are shown in Figures 5−10, together with the curves of the corresponding surfactants into water without DNA for comparison. All of the calorimetric titration curves have been corrected by the subtraction of the DNA dilution enthalpy during the above titration process.

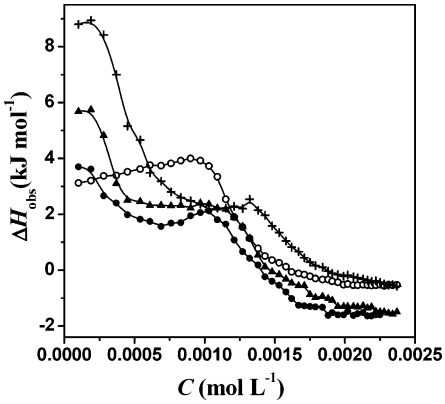

Figure 7. Calorimetric titration curves of $C_{12}C_6C_{12}Ac_2$ into water (○), 0.33 mM DNA (●), 0.65 mM DNA (▲), and 1.63 mM DNA (+) at 298.15 K.

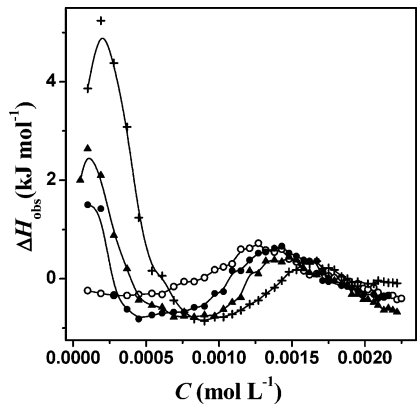

Figure 8. Calorimetric titration curves of $C_{12}C_6C_{12}Cl_2$ into water (○), 0.33 mM DNA (●), 0.65 mM DNA (▲), and 1.63 mM DNA (+) at 298.15 K.

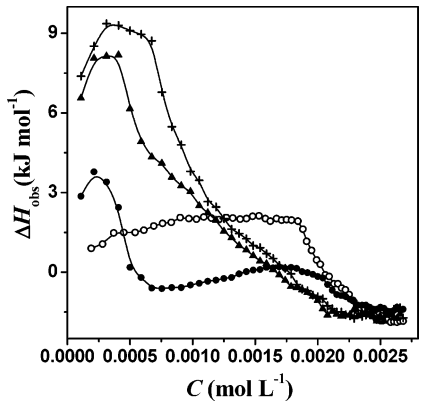

Figure 9. Calorimetric titration curves of $C_{12}C_6C_{12}F_2$ into water (○), 0.33 mM DNA (●), 0.65 mM DNA (▲), and 1.63 mM DNA (+) at 298.15 K.

The titration curves in DNA solution for different surfactants with monovalent counterions become increasingly different from the characteristic sigmoidal shape seen for the surfactant in the absence of DNA. The differences can be directly attributed to interactions between DNA and surfactants. The titration process into DNA basically changes from the typically large endothermic enthalpy of dilution to small endothermic or slightly exothermic enthalpy, and eventually the ΔH_{obs} are all small. In the first few injections, ΔH_{obs} is more positive in DNA solution compared to the dilution curve in water, due to the breakup of surfactant micelles and endothermic interactions of DNA with surfactant monomers. Then, the initial stage of titrations is

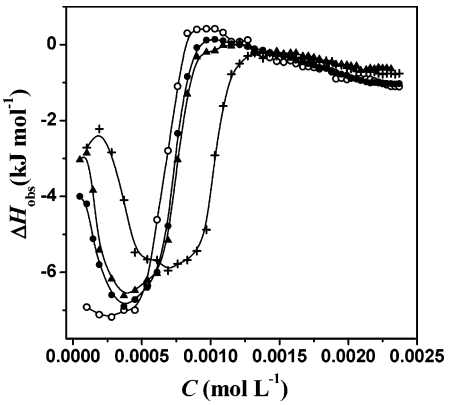

Figure 10. Calorimetric titration curves of $C_{12}C_6C_{12}SO_4$ into water (○), 0.33 mM DNA (●), 0.65 mM DNA (▲), and 1.63 mM DNA (+) at 298.15 K.

accompanied by formation of DNA−surfactant monomer aggregates, justified by the turbidity titration experiments conducted under the same conditions as those used in the calorimetric experiments. In the turbidity titration experiments, with the initial addition of surfactant solution into DNA, the turbidity increases slowly and the solution becomes turbid. However, no precipitate was observed during the entire titration process. Thus, the DNA−surfactant aggregates could be thought as a dispersed solid state rather than as a precipitate. Due to the complicated and intriguing phase behavior of the gemini DNA−surfactant systems, further detailed study is in process. With the further addition of surfactant solution, micelle-like surfactant aggregates start to form on DNA chains, which is not completely the same as the micelles in the absence of DNA. Correspondingly, ΔH_{obs} begins to decrease. Here, the concentration at which DNA−surfactant micelle aggregates start to form is defined as the critical aggregation concentration (*CAC*). Beyond *CAC*, ΔH_{obs} decreases sharply and becomes exothermic relative to the dilution curve in water, which can be ascribed to the exothermic interactions between DNA and surfactant micelles. The value of ΔH_{obs} then varies slowly, sometimes with the appearance of a plateau, until the second critical concentration (C_2) is approached, at which all DNA is saturated by bound surfactant molecules. After this point, free micelles start to appear and the further addition of surfactant will only lead to dilution of the micellar solution, the result of this is that the curve coincides with the dilution curve of surfactant without DNA. Finally, ΔH_{obs} becomes quite small and almost close to zero.

For $C_{12}C_6C_{12}SO_4$, the behavior is different. The titration process now changes in the opposite direction (from exothermic to endothermic), as in water alone. The more positive ΔH_{obs} generated in the first few injections indicates endothermic interactions between $C_{12}C_6C_{12}SO_4$ and DNA. As aggregates start to form at the *CAC*, ΔH_{obs} decreases rapidly followed by a steep increase, and then the process becomes exothermic relative to the dilution curve in water until C_2 is reached. Eventually, as for the other counterions, the dilution curve in the DNA solution should join the one in water and the curve levels off to a small value of ΔH_{obs}.

Values of the *CAC*, C_2, the enthalpies of aggregation (ΔH_{agg}), the Gibbs free energies of aggregation (ΔG_{agg}), and the entropies of aggregation (ΔS_{agg}) for the $C_{12}C_6C_{12}X_2$ into DNA obtained from the calorimetric measurements are summarized in Table 2. All of the thermodynamic quantities are expressed per mole of surfactant. The values of the *CAC* region are very low so that the *CAC* cannot be determined precisely, but the values are useful for comparison purposes. The value of C_2 is determined approximately as the concentration where the titration curve in the presence of DNA joins the dilution curve in the absence of DNA, where allowance sometimes has to be made for small calibration errors at zero ΔH_{obs}. This method of determining C_2 will be an overestimate because the curves will actually merge at a slightly higher concentration than where free micelles start to form. The value of ΔH_{agg} was determined by the same procedure as the ΔH_{mic}, described above. The value of ΔG_{agg} can be calculated from the *CAC* and β' using an equation similar to that for ΔG_{mic}. The quantity β' is the degree of association of the counterion with the DNA−surfactant aggregate. This is assumed to be equal to β for the free micelle, although there is some uncertainty in this assumption.[35,52] The value of ΔS_{agg} can be derived from ΔH_{agg} and ΔG_{agg} in the usual manner.

TABLE 2: Critical Aggregation Concentration (*CAC*), Saturation Concentration (C_2), and Thermodynamic Parameters for the $C_{12}C_6C_{12}X_2$ Diluted into Various Concentrations of DNA at 298.15 K from Calorimetric Measurements

	DNA (mmol L^{-1})	CAC (mmol L^{-1})	C_2 (mmol L^{-1})	ΔH_{agg} (kJ mol^{-1})	ΔG_{agg}[a] (kJ mol^{-1})	$T\Delta S_{agg}$[b] (kJ mol^{-1})	ΔH_{DS} (kJ mol^{-1})	ΔG_{DS} (kJ mol^{-1})	$T\Delta S_{DS}$ (kJ mol^{-1})
SO_4^{2-}	0.33	0.10	1.1	6.3 ± 0.4	−36.5	42.8	−1.0	−6.6	5.6
	0.65	0.11	1.3	5.8 ± 0.4	−36.1	41.9	−1.5	−6.2	4.7
	1.63	0.20	1.4	5.3 ± 0.5	−33.8	39.1	−2.0	−3.9	1.9
NO_3^-	0.33	0.10	0.9	−7.7 ± 0.5	−47.7	40.0	−1.2	−11.4	10.2
	0.65	0.11	1.0	−9.3 ± 0.5	−47.2	37.9	−2.8	−10.9	8.1
	1.63	0.19	1.2	−11.0 ± 0.4	−44.3	33.3	−4.5	−8.0	3.5
Br^-	0.33	0.19	0.9	−8.9 ± 0.3	−43.9	35.0	−3.8	−8.3	4.5
	0.65	0.20	1.0	−9.6 ± 0.8	−43.6	34.0	−4.5	−8.0	3.5
	1.63	0.23	1.2	−11.9 ± 0.8	−42.9	31.0	−6.8	−7.3	0.5
Ac^-	0.33	0.20	1.0	−5.1 ± 0.3	−42.8	37.7	−0.3	−8.5	8.2
	0.65	0.23	1.2	−7.2 ± 0.2	−42.1	34.9	−2.4	−7.8	5.4
	1.63	0.26	1.3	−8.7 ± 0.2	−41.4	32.7	−3.9	−7.1	3.2
Cl^-	0.33	0.20	1.3	−1.9 ± 0.4	−40.9	39.0	−1.0	−9.3	8.3
	0.65	0.30	1.4	−3.3 ± 0.6	−38.9	35.6	−2.4	−7.3	4.9
	1.63	0.40	1.5	−5.3 ± 0.4	−37.4	32.1	−4.4	−5.8	1.4
F^-	0.33	0.21	2.0	−5.0 ± 0.3	−36.8	31.8	−0.9	−8.4	7.5
	0.65	0.39	2.2	−9.6 ± 0.5	−35.8	26.2	−5.5	−7.4	1.9
	1.63	0.68	2.3	−10.2 ± 0.6	−33.1	22.9	−6.1	−4.7	−1.4

[a] Calculated using $\Delta G_{agg} = RT(1 + 2\beta')\ln cac - RT\ln 2$ and $\Delta G_{agg} = RT(1 + \beta')\ln(cac/2)$ for gemini surfactants with monovalent counterions and with divalent counterion, respectively,[50] where *cac* is expressed in molarity of each alkyl chain, twice as much as the *CAC* expressed in the molarity of surfactant; it was assumed that $\beta' = \beta$, and β was obtained according to the relation $\beta = 1 - \alpha$. [b] Calculated from $\Delta G_{agg} = \Delta H_{agg} - T\Delta S_{agg}$. [c] ΔH_{DS}, ΔG_{DS}, and $T\Delta S_{DS}$ are calculated by $X_{DS} = X_{agg} - X_{mic}$.

1. Dependence on the Nature of the Counterion. The results in Table 2 show that the *CAC* is 2 to 6 times lower than the corresponding *CMC*, indicating that interaction between DNA and these surfactants starts at very low surfactant concentrations. Despite the difficulty of determining the *CAC* accurately because of its early occurrence in the titration curves, the values of the *CAC* at a given DNA concentration vary in the order of $SO_4^{2-} < NO_3^- < Br^- < Ac^- < Cl^- < F^-$, exactly the same as the *CMC*s in the absence of DNA. However, whereas the enthalpies of micellization of the monovalent anions also followed the same order as the *CMC*s, apart from the anomalous chloride, the pattern of the behavior of both the enthalpy of aggregation and the thermodynamic properties of binding is less obvious.

The aggregation process can be thought of as consisting of a contribution from micellization and a contribution from the binding of the micelle to DNA. The thermodynamic properties of binding, for example, ΔG_{DS}, can be obtained from the differences of $\Delta G_{agg} - \Delta G_{mic}$. The values of ΔG_{DS} are all negative, confirming the strong binding of the micelles to DNA, but there is no obvious pattern that correlates with the Hofmeister series. The contribution from micellization should parallel that for micellization in the absence of DNA. The contribution from micelles binding to DNA will depend mainly on electrostatic interactions, that is, the degree of ionization of the micelle and the binding strength of the counterions to the micelle and to DNA. Displacement of counterions could be endothermic or exothermic, depending on changes in hydration, but will generally be expected to be accompanied by a gain in entropy. Comparison of the enthalpies and entropies of aggregation with those of micellization indicates that the enthalpies ΔH_{DS} are generally small and exothermic, but the entropies are all large at typical $T\Delta S_{DS}$ values of 10 kJ mol^{-1}. Thus, the binding of the micelles to DNA is more or less completely determined by the large gain in entropy resulting from release of the counterions. This explains why the binding of the micelles to DNA is the weakest for the sulfate complex, because only half the number of ions is released from the micelle. Also, among the monovalent ions the highest gain of entropy is for the least dissociated micelle, that of the nitrate ion. The pattern of free energies of binding in the monovalent ion series follows the Hofmeister series, except for the bromide system, which is out of place. This is the only system for which the enthalpy of binding to DNA is significantly above the experimental error and it is distinctly exothermic even at the lowest DNA concentration. The gain in entropy is also unusually low in comparison with the rest of the series. These two parameters seem to have combined to displace the bromide system from its place in the Hofmeister series. It is also interesting that, although the enthalpy of micellization for the chloride system was anomalous, its binding to DNA is much more closely in line with that expected from the Hofmeister series. Indeed, apart from the bromide, the binding of the other four monovalent systems follows the Hofmeister series exactly.

2. Dependence on DNA Concentration. The calorimetric titration experiments were carried out at three DNA concentrations, 0.33, 0.65, and 1.63 mM phosphates.

The titration curves at various DNA concentrations for each of the $C_{12}C_6C_{12}X_2$ into DNA at 298.15 K are generally similar in shape and, in all cases, the exothermic peak shifts to higher surfactant concentration as the DNA concentration increases, showing that there is an increase in the interaction with DNA with increasing concentration. However, the results in Table 2 show that the increase in both the *CAC* and C_2 is not linear in DNA concentration, suggesting that there may be a degree of cross linking of the DNA by the attached micelles, that is, the degree of attachment of DNA to the micelles increases with DNA concentration. As the DNA concentration increases, ΔG_{agg} becomes less negative and ΔH_{agg} becomes more negative, but in all cases $T\Delta S_{agg} > -\Delta H_{agg}$, showing that the aggregation of the $C_{12}C_6C_{12}X_2$ with DNA is driven by entropy in all the circumstances investigated here.

Conclusions

In this study, we report the results of the first systematic investigation of the effect of counterions on the micellization of the $C_{12}C_6C_{12}X_2$ gemini surfactants in aqueous solution as well as on their interaction with DNA. The various thermodynamic parameters of the two processes have been obtained from the results of isothermal titration microcalorimetry and conductivity measurements. The values of enthalpy changes for dilution of these surfactants with monovalent counterions into pure water and into DNA are all negative, whereas those for $C_{12}C_6C_{12}SO_4$ are all positive. This is interpreted tentatively as being associated with changes of hydration during the association. The counterion has a marked influence on both micellization and aggregation. The *CMC*, *CAC*, and free energies of aggregation generally change closely parallel the Hofmeister series, but the pattern of behavior of the enthalpies is often more complex, revealing features that are probably associated with different levels of hydration. The binding of micelles to DNA is dominated by the large gain in entropy on release of the small counterions from the micelles and DNA.

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